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TI Inhibition of viral and cellular promoters by human wild-type p53
AU Subler, M. A.; Martin, D. W.; Deb, S.
SO Journal of Virology (1992), 66(8), 4757-62
What promoter drives p53 expr??

TI Oncogenes and tumor suppressor genes regulate the human multidrug resistance gene (MDR1) expression
AU Kim, Sun Hee; Park, Yeung Hong; Kim, Dong Wan; Kang, Chi Dug; Chung, Byung Seon
CS Coll. Med., Pusan Natl. Univ., Pusan, 602-739, S. Korea
SO Molecules and Cells (1993), 3(1), 13-16
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TI Differential effect of p53 on the promoters of mouse DNA polymerase .beta. gene and proliferating-cell-nuclear-antigen gene
AU Yamaguchi, Masamitsu; Hayashi, Yuko; Matsuoka, Shuhei; Takahashi, Takashi; Matsukage, Akio
SO European Journal of Biochemistry (1994), 221(1), 227-37
What promoter drives p53 expr??

TI Wild-type human p53 transactivates the human proliferating cell nuclear antigen promoter
AU Shivakumar, Chittari V.; Brown, Doris R.; Deb, Sumitra; Deb, Swati Palit
SO Molecular and Cellular Biology (1995), 15(12), 6785-93
What promoter drives p53 expr??

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Oncogenes and Tumor Suppressor Genes Regulate the Human Multidrug Resistance Gene (MDR1) Expression

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The regulation of the *MDR1* gene by the expression of oncogenes or tumor suppressor genes was examined. We found that the *MDR1* promoter is remarkably activated by the expression of *c-raf* gene but is suppressed by the expression of *c-myc* or H-*ras* gene in transient expression assay. These results were in good agreement with those obtained from Northern blot analysis in mouse leukemia P388 cell line and its multidrug resistant subline. In addition, the expression of *Rb* or *p53* gene increased the *MDR1* promoter activity. These results suggest that the acquisition of MDR phenotype by tumor cells may be associated with the alterations in the expression level of oncogenes and tumor suppressor genes.

The development of acquired multidrug resistance (MDR) is one of the major problems in cancer chemotherapy (Nooter *et al.*, 1991). Many MDR cells, which exhibit resistance to structurally and functionally unrelated agents, express increased level of a 170-kDa P-glycoprotein encoded by the *MDR1* gene (Goldstein *et al.*, 1989). Recent studies demonstrate that the acquisition of MDR phenotype by tumor cells is associated with the loss of tumorigenicity (Delaporte *et al.*, 1988; Larsen *et al.*, 1989). The MDR clone of chinese hamster lung cells showed decreased level of *c-myc* gene copy number and expression as compared to drug-sensitive parental cells (Delaporte *et al.*, 1991). The increased numbers of EGF receptor were also observed in MDR cell lines derived from mouse and human cell lines. These MDR clones manifest a more normalized phenotype and a lower tumorigenic potential, and the increased expression of EGF receptor in MDR cells might be associated with reverse transformation (Biedler *et al.*, 1988). In addition, the expression of N-*myc* was thought to be correlated with the down-regulation of the *MDR1* expression in human neuroblastomas (Takano *et al.*, 1989). The decreased tumorigenicity observed in several MDR cell lines suggested that at least in some cases, development of drug resistance may be associated with the alteration of certain oncogenes or tumor suppressor genes.

In this report, as an approach to understand the relationship between the acquisition of MDR phenotype by tumor cells and alteration in oncogenes or tumor suppressor genes, we examined the effects of these genes on the *MDR1* promoter activity using transient expression assay and the expression of oncogenes in MDR clone derived from P388 and FM3A cell lines.

Materials and Methods

Cells and Plasmids

African green monkey kidney cell line CV-1 and golden hamster embryo lung cell line GHE-L were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum. Mouse leukemia cell line P388, mouse mammary carcinoma cell line FM3A and these MDR cells were cultured in suspension in RPMI 1640 medium supplemented with 10% fetal calf serum. An MDR subline of P388, FM3A was obtained by stepwise selection in colchicine, MDR subline of P388 (P388/M) exhibited 224-fold resistance to colchicine and 76-, 29- and 30-fold cross resistance to vinblastin, adriamycin and vincristine, respectively, MDR subline of FM3A (FM3A/M) exhibited 78-fold resistance to colchicine and 42-, 13-, and 43-fold cross resistance to vinblastin, adriamycin, and actinomycin-D, respectively. The plasmid expressing H-*ras*, *c-fos*, *Rb* and *p53* genes were constructed by cloning each coding region under EF-α promoter of pEF321 expression vector (Kim *et al.*, 1990). The plasmid pSVc-*myc* contains human *c-myc* gene under the SV40 early promoter and the plasmid pCORAF contains the cDNA of human activated *c-raf*-1 protein cloned into pCO12 expression plasmid (Kaibuchi *et al.*, 1989). The plasmid p970MDR-CAT, which contains promoter region (970-bp *Pst*I fragment) of human *MDR1* gene and bacterial CAT gene, obtained from Sugimoto Y. of Japanese Foundation of Cancer Research (Ueda *et al.*, 1987).

DNA transfection and CAT assay

Subconfluent cultures of GHE-L cells or CV-1 cell

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lines were cotransfected with 10 μg of pMDRCAT and 10 μg of plasmid indicated in each experiment by the calcium phosphate coprecipitation method (Graham *et al.*, 1973). The DNA precipitates were applied to the cells for 6 h, and the cells were treated with growth medium containing 20% DMSO for 3 min. CAT assays were performed according to the method of Gorman (Gorman *et al.*, 1982). After 48 h transfection the cells were washed with PBS and resuspended in 200 μl of 1.25 M Tris-HCl (pH 7.8) and disrupted by six cycles of freeze-thawing. The protein content in cell lysate was determined by the method of Bradford (Bradford *et al.*, 1976). An aliquot of the lysate was incubated with 0.2 μCi[^{14}C] chloramphenicol and 1 mM acetyl CoA in 0.25 M Tris-HCl (pH 7.8) at 37 ℃ for 4 h. The reaction products were separated by ascending thin-layer chromatography using chloroform and methanol (95:5).

Northern blot analysis

Total cytoplasmic RNA was extracted according to the procedure described by Maniatis *et al.* (1989). Denatured RNA samples (20 μg/well) were fractionated by electrophoresis in 1% agarose gel containing 7% formaldehyde and transferred onto a nylon membrane. The membrane was prehybridized at 42 ℃ for 4 h in the presence of 50% formamide, 0.1% sodium pyrophosphate, 0.2% SDS and hybridized at 42 ℃ for 20 h in the same buffer containing the ^{32}P-labeled probe. After washing with 0.1% SDS and 0.1× SSC, the membrane was subjected to autoradiography.

Result and Discussion

The development of resistance to various chemotherapeutic drugs in tumor cells results in decreased tumorigenicity and less malignant phenotype relative to the drug-sensitive parental cells (Biedler *et al.*, 1988). It is known that the acquisition of MDR phenotype by tumor cells may be correlated with the alteration of oncogenic potential (Takano *et al.*, 1989).

To examine the effects of oncogenes or tumor suppressor genes on *MDR1* promoter activity, plasmids containing oncogenes or tumor suppressor genes were cotransfected with p970MDRCAT plasmid to GHE-L cells and the promoter activity was estimated by CAT assay. As shown in Figure 1A, the products of H-*ras*, c-*myc* or c-*fos* gene appeared to suppress the *MDR1* promoter. In contrast, Chin *et al.* (1992) have reported that the activity of MDR1 promoter could increase by c-Ha-*ras*. This difference would be due to the fact that the human *MDR1* gene promoter used by Chin *et al.* (1992) contains the 1.8-kb geomic sequence upstream from the initiation codon (ATG) of the human *MDR1* gene, whereas p970MDR-CAT used in this experiment contains only 970-bp promoter region (−433 to +547) of the human *MDR1* gene. Under the same condition, the *MDR1* promoter was significantly activated by the expression of c-*raf* gene. But unexpectedly, the expression of tumor suppressor gene *Rb* resulted in a slight increase in *MDR1* promoter activity

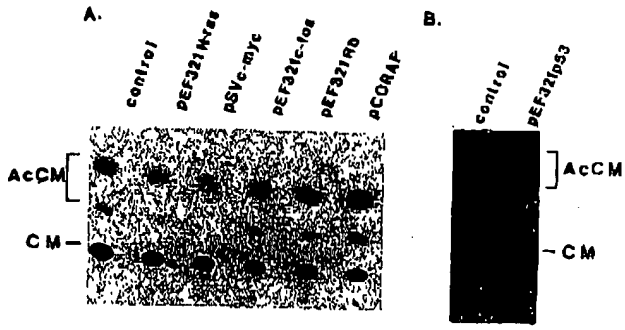

Figure 1. Modulation of *MDR1* promoter activity by the expression of oncogenes or tumor suppressor genes in GHE-L cells. The reporter gene, p970MDRCAT, was cotransfected with indicated plasmid. p970MDRCAT alone was used as a control. CAT activity was estimated 48 h after transfection. CM and AcCM represent chloramphenicol and its acetylated forms, respectively.

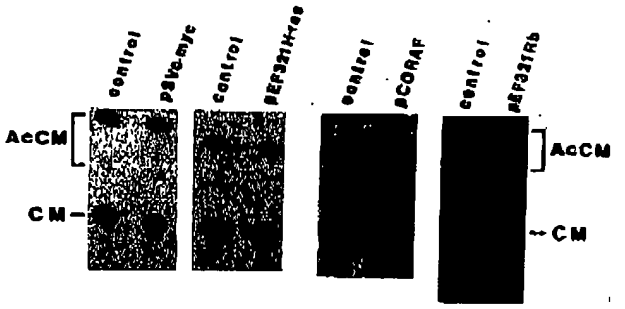

Figure 2. Modulation of *MDR1* promoter activity by the expression of oncogenes or tumor suppressor gene in CV-1 cells. The transfection and assay of report gene activity were as described in the legend of Figure 1.

(Fig. 1A), and the expression of another tumor suppressor gene *p53* significantly activated the *MDR1* promoter (Fig. 1B). The similar effects of oncogenes and tumor suppressor genes on the *MDR1* promoter activity were also observed in CV-1 cells showing the suppressive activity of c-*myc* or H-*ras* and promoting activity of *Rb*. The c-*raf* gene activated significantly the *MDR1* promoter even in CV-1 cells (Fig. 2). These results suggest that the *MDR1* promoter activity may be regulated by oncogenes and tumor suppressor genes.

In further study, we isolated MDR cells which exhibit cross-resistance to colchicine, vinblastine, adriamycin and vincristine, from mouse leukemia P388 cells. Relative resistance for each drug is 224, 76, 29 and 30-fold as compared with parental P388 cells, respectively. By Northern blot analysis, the 4.5 kb *MDR1* transcript was detected in the isolated MDR cells (data not shown) but was not detectable in parental P388 cells indicating that the acquired MDR phenotype is mainly due to the increased expression of *MDR1* gene.

Then we compared the amount of c-*myc* and c-*raf* transcripts between parental P388 cells and isolated MDR cells. As shown in Figure 3, high level of c-*myc* transcript was detected in parental P388 cells (P 388/S), but this transcript was remarkably decreased in its MDR cells (P388/M). The similar result showing the decreased expression of c-*myc* gene in MDR cells was previously reported in chinese hamster lung cell line DC-3F (Delaporte *et al.*, 1991). By contrast, the level of c-*raf* transcript was higher in MDR cells than in parental P388 cells (Fig. 4). The similar result was also observed in MDR cells isolated from FM3A cells. The isolated MDR cells exhibited cross-resistance to colchicine, vinblastine, adriamycin and actinomycin D. Relative resistance for each drug is 78-, 42-, 13- and 43-fold as compared with parental FM3A cell. Also expression of *MDR1* gene was observed (data not shown). The c-*raf* gene was not expressed in parental FM3A cells (FM3A/S), whereas was actively transcribed in its MDR cells (FM3A/M) (Fig. 4). The results suggest that the changes in the level of oncogene expression may affect the transcription level of *MDR1* gene, although the pathway is not known.

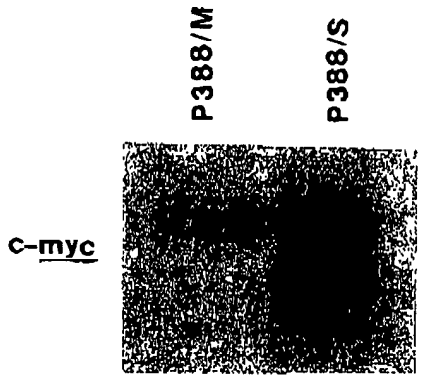

Figure 3. Northern blot analysis of oncogenes expressed in multidrug-resistant and parental P388 cells. Cytoplasmic RNA (20 μg) obtained from the individual cell line were hybridized with the ^{32}P-labeled cDNA of c-*myc* gene.

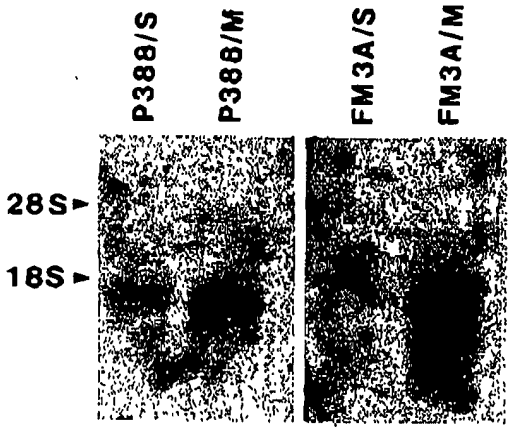

Figure 4. Northern blot analysis of c-*raf* RNA molecule expressed in multidrug-resistant and parental mouse tumor cells. RNA (20 μg) obtained from the individual cell line were hybridized with the ^{32}P-labeled cDNA of c-*raf* gene.

The c-*raf* protein is known from its structural and biochemical features to be a cytoplasmic serine/threonine protein kinase (Bonner *et al.*, 1986) and it mediates the signals transmitted by PDGF, EGF and protein kinase C from the plasma membrane to the cytoplasm (Wasylyk *et al.*, 1989). But the mode of action of the c-*raf* protein in activating *MDR1* promoter is not known. Interestingly, the products of *p53* or *Rb* genes which are nuclear tumor suppressor proteins activated the *MDR1* promoter. The *p53* protein binds to the SV40 large T antigen and inhibits its oncogenic potential (Cathy *et al.*, 1989). It may be noteworthy that *MDR1* promoter is markedly activated by the expression of *p53* gene (Fig. 1B) but is suppressed in about one-fifth by the expression of SV40 large T antigen gene (data not shown).

Tumor cells have shown their numerous genetic alterations including activation of oncogenes and inactivation of tumor suppressor genes. Among the oncogenes, *myc* and *ras* genes appeared to play a central role in many types of tumors (Cathy *et al.*, 1983). Our results show that the *MDR1* promoter is markedly suppressed by the expression of c-*myc* or H-*ras* gene. They also suggest that the acquisition of MDR phenotype by tumor cells may be derived from the down-regulated expression level of oncogenes and increased expression of tumor suppressor genes. Further studies are required to clarify the detailed mechanism of regulation of *MDR1* gene expression by c-*myc* or H-*ras* oncogenes or tumor suppressor genes.

Acknowledgments

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